

(dhfr<sup>-</sup>) were mixed therewith to be the concentration of  $1 \times 10^5$  cell/ml, and they were inoculated into the dishes of 60mm diameter and were cultured for 24 hours under the condition of 37°C and 5 % CO<sub>2</sub>. Culture supernatant was discarded, then IMDM containing 10% FCS were added to be 6 ml wherein IMDM contains 100  $\mu$ l of solution prepared by mixing 5  $\mu$ g of DNA (Expression Vector pNOW1-hMBP) with lypofectin solution (DOTAP Liposomal Transfection Reagent; Boehringer Mannheim), and Expression Vector pNOW1-hMBP was introduced into the host CHO cells of dhfr<sup>-</sup> by further adding thereto hypoxanthine (final concentration of 10nM) (GIBCO) as well as thymidine (final concentration of 100nM) (GIBCO) and culturing it for 16 hours. After then, culture supernatant was discarded, then 6 ml of IMDM supplemented therein 10% FCS, hypoxanthine and thymidine are added thereto, and the culture were continued for another 24 hours.

## (2) Production of Neomycin (G418) Resistance CHO Cells

After 24 hours culture, the cells introduced thereinto the expression vector pNOW1-hMBP, such cells were treated with trypsin, then were collected from dishes, and were counted on the cell numbers, thereafter, cell suspension were inoculated (poured) into 10 pieces of 96-well microplate in the amount of 0.1ml/well wherein the cell suspension is suspended by IMDM supplemented thereto 10% FCS and contained 400  $\mu$ g/ml of Neomycin (G418) to be the concentration of  $1 \times 10^5$  cell/ml. When the culture had been